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Rick R. Wascher — Printed Name

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Markov, et al.

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Title: METHOD AND APPARATUS FOR DETERMINING BIOLOGICALLY USEFUL FIELD METRICS ASSOCIATED WITH MAGNETIC FIELDS

Commissioner of Patents and Trademarks
Washington, D.C. 20231

INFORMATION DISCLOSURE STATEMENT

Dear Sir:

This Information Disclosure Statement is being submitted in accordance with Applicant's duty of disclosure under 37 C.F.R. § 1.97. Attached hereto and listed on the accompanying Form PTO-1449 is a list of the references of which Applicant is aware and are potentially relevant to the examination of the above-referenced case. Appendix A, attached (i.e., stapled) hereto, is a summary of the relevance of each of the references cited on Form PTO-1449.

Remarks

Appendix A is attached and is intended to accompany this page. Appendix A contains the summary of each of the items submitted pursuant to this disclosure statement as listed on Form PTO-1449. None of the references by themselves or combined in any way would preclude a patent issuing from the above-referenced application.

Respectfully submitted,

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Appendix A

Alternate Binding of Actin and Calmodulin top Multiple Sites on Dystrophin

These results suggest that calmodulin (or troponin C) binding alternates with and may regulate F-actin binding by dystrophin much as has been postulated for other cytoskeletal proteins which are homologous to dystrophin.

Oxygen Effect in the Radiolysis of Proteins

Radiolysis of myoglobin was carried out under air and under nitrogen in phosphate buffer at pH 5 and 7. The radiation products were separated by SDS-polyacrylamide gel electrophoresis and by HPL gel chromatography with guanidine HCl. Under nitrogen the main reaction is the aggregation caused by covalent cross-links. Under air the radiolysis leads to peptide chain scission, which is not a random process, but produces specific protein fragments. The estimated molecular weights of these fragments gave further support to the assumption that the aminoacyl-proline peptide group is the preferential breaking site. In contrast to haemoglobin, myoglobin showed nearly no radiation-induced fragmentation under nitrogen.

Relationships Between Muscle Membrane Lipids, Fiber Type, and Enzyme Activities In Sedentary and Exercised Rats

Taken together, these results suggest that diet and exercise may improve insulin action through separate and synergistic mechanisms.

Multiple Growth Factors are Released from Mechanically Injured Vascular Smooth Muscle Cells

In this study, we demonstrated by immunoblot analysis and antibody neutralization studies that mechanically injured vascular VSMC immediately release the biologically active growth factors bFGF, PDGF, and EGF. Although these factors are released in small quantities, they probably interact synergistically to produce a significant mitogenic signal. Therefore, release of multiple growth factors due to mechanical injury may be an important early mitogenic stimulus for VSMC proliferation.

Thiophosphorylation Independently Activates Each Head of Smooth Muscle Myosin In Vitro

These results suggest that, under our assay conditions, LC_{20} thiophosphorylation activates motor and biochemical properties of a single myosin head, independent of the phosphorylation state of its neighboring head.

Ca^{2+} Currents in Human Colonic Smooth Muscle Cells

These results suggest that the fast-inactivating (transient) current component might be a T-type Ca^{2+} current ($I_{Ca,T}$), whereas the sustained component is an L-type Ca^{2+} current ($I_{Ca,L}$). The current density of total I_{Ca} (L+T), $I_{Ca,L}$, and $I_{Ca,T}$ declined with aging, but the decline in $I_{Ca,T}$ was less.

Modulation of Smooth Muscle Contraction by Sphingosylphosphorylcholine

This study demonstrates that SPC is important in cellular signaling of gastrointestinal smooth muscle cells through a mechanism that is independent of IP_3-sensitive calcium release and probably through activation of a protein kinase C-mediated activation of MAP kinase.

Comparative Studies of the Monomeric and Filamentous Actin-Myosin Head Complexes

Taken together, the results suggested that the G-actin--SS1 interaction encompasses only a small fraction of the strong as well as of the weak F-actin--S1 interface. The monomeric complex would in fact resemble more the collision complex which takes place early in the F-actin--S1 interaction.

Functional Significance of the Binding of One Myosin Head to Two Actin Monomers

These results implied that the activation of SA ATPase by actin requires the interaction of S1 with a second actin monomer within the thin filament. They also suggested that the full activation of the filamentous complex is not dependent on the degree of saturation of the thin filament by myosin.

Effects of CGS 9343B (a Putative Calmodulin Antagonist) on Isolated Skeletal Muscle

These data demonstrate that: 1) hexose transport, both in the absence and presence of external stimuli (insulin and hypoxia), requires functional calmodulin; and 2) insulin-mediated activation of glycogen synthase does not require functional calmodulin, nor can it be accounted for by increases in glucose transport or glucose-6-P.

Proposed Mechanism of Force Generation in Striated Muscle

Recordings of the change in tension in striated muscle after a sudden alteration of the length have made it possible to suggest how the force between the thick and thin muscle filaments may be generated.

The Calmodulin Binding Domain of Chicken Smooth Muscle Myosin Light Chain Kinase Contains a Pseudosubstrate Sequence

In this paper we define a smooth muscle calmodulin-binding sequence and show that synthetic peptides corresponding to both the skeletal and smooth muscle calmodulin binding regions are also potent competitive inhibitors with respect to substrate.

Some Thoughts Regarding EF-hand and the Structure of Calbinding

We attempt here to answer this question, as well as to examine some of the ways in which EF-hand proteins have diversified and to speculate concerning possible variations yet to be discovered on the EF-hand theme.

Pre-Steady-State Kinetics of the Activation of Rabbit Skeletal Muscle Myosin Light Chain Kinase by CA^{2+}/Calmodulin

Our results also indicated that kinase activity occurred too rapidly for the slower isomerization rate of 2.2 s^{-1} to be linked specifically to the activation process.

Calcium Binding to Metallochromic Dyes and Calmodulin in the Presence of Combined, AC-DC Magnetic Fields

The possibility that weak, ac and dc magnetic fields in combination may affect binding equilibria of calcium-ions (Ca^{2+}) was investigated with two metallochromic dyes as calcium-binding molecules; murexide and arsenazo III. Calcium-dye equilibria were followed by measuring solution absorbances with a fiber-optic spectrophotometer. A Ca^{2+} -arsenazo solution was also used indirectly to monitor the binding of Ca^{2+} to calmodulin. Parallel, ac and dc magnetic fields were applied to each preparation. The ac magnetic field was held constant during each of a series of experiments at a frequency in the range between 50 and 120 Hz (sine wave) or at 50 pps (square wave) and at an rms flux density in the range between 65 and 156 μT. The dc magnetic field was then varied from 0 to 299 μT at 1.3 μT increments. The magnetic fields did not measurably affect equilibria in the binding of metallochromic dyes or calmodulin to Ca^{2+}.

Structural Studies on Calmodulin and Troponin C: Phenothiazine, Peptide, and Protein Interactions with Calcium-Induced Helices

The emphasis in this chapter stresses the structural information on the interactions between CaM and TnC with synthetic and peptide antagonists and proteins and the location of these interactions within the sequence of these homologous proteins.

Calmodulin and Its Role in the Second-Messenger System

The present chapter is written for the general reader, to provide an overview of, and also to highlight, current studies of this protein. The review of the literature will be selective rather than comprehensive. Most of the data used for illustrative purposes are from the authors' laboratory.

^{1}H NMR Studies of Calmodulin-Peptide Interactions

We report here the results of NMR studies of five CaM-binding peptides aimed at determining the existence and nature of peptide-induced conformational changes in CaM.

Calmodulin Antagonists and Cellular Physiology

The purpose of this chapter is not to provide an extensive review of the biochemical mechanisms by which Ca^{2+} may regulate smooth muscle contraction, for which the reader may refer to previously cited reviews. Instead, this chapter will focus on the general properties of the myosin phosphorylation system as a calmodulin-regulated system.

Activation of Rabbit Skeletal Muscle Myosin Light Chain Kinase by Calmodulin – a Mechanistic Overview

The myosin light chain kinases (MLCK, E.C. 2.7.1.37) are a family of tissue-specific isozymes which share as their substrate the phosphoryl- or P-light chain component of myosin. Distinct forms of MLCK have been isolated from smooth, skeletal, and cardiac muscle. Catalytically, although skeletal muscle MLCK will phosphorylate P-light chains from all tissue sources with high efficiency in vitro, smooth muscle MLCK will only phosphorylate smooth muscle myosin P-light chains (43).

The Linker of Calmodulin – To Helix or Not To Helix

The linker regions of the central helices of calmodulin and of troponin C are observed to be α-helices in crystal and in solution. However, these linkers are predicted to be non-helical by standard algorithms. Further, there is strong evidence that when calmodulin interacts with some of its targets this linker helix bends. The linker appears to be delicately balanced between helical and non-helical conformations. A review of this subject suggests that one can anticipate more unpredicted conformations for the central helices on the score of other proteins that have four EF-hand domains.

Calmodulin, Cell Growth and Gene Expression

Calmodulin is thought to regulate a number of intracellular processes, including cell proliferation. Previous studies using drugs that antagonize calmodulin function have indicated that calmodulin is required for progression at specific points in the eukaryotic cell cycle. However, interpretation of these results has previously been difficult due to the lack of specificity of these inhibitors in living cells. Recent studies have used a combination of molecular biological and genetic analysis techniques to approach the study of calmodulin-dependent cell cycle control with greater precision and specificity. These studies have confirmed that calmodulin is an important regulator of the cell cycle, and provide new ways in which to examine the cellular mechanisms involved in calmodulin-dependent cell cycle control.

Calmodulin Regulation of Smooth-Muscle Myosin Light-Chain Kinase

Calmodulin is the predominant Ca^{2+} receptor in all nonmuscle and smooth muscle cells. As such, it mediates the activity of more than 20 intracellular enzymes. The structure of calmodulin reveals an eight-turn helix that separates the two pairs of Ca^{2+} binding sites. This central region is involved in enzyme recognition and or activation. Now that the sequence of several calmodulin-dependent enzymes is known, it has been revealed that the sequence of each calmodulin binding region is unique but bind calmodulin with equal affinity. The amino-terminal portion of this region in calmodulin-dependent protein kinases serves to inhibit the enzyme from binding substrate in the absence of calmodulin. This pseudosubstrate region is both unique and specific for each enzyme. Therefore calmodulin derepresses rather than activates protein kinases. Studies with smooth-muscle myosin light-chain kinase have identified the pseudosubstrate region. It is proposed that inhibitors directed toward this intramolecular interaction should provide novel drugs for the treatment of a variety of cardiovascular diseases that result in the elevated systemic vascular resistance.

The Activity of Calmodulin is Altered by Phosphorylation: Modulation of Calmodulin Function by the Site of Phosphate Incorporation

These results demonstrate that phosphorylation is an *in vitro* regulatory mechanism in the targeting of calmodulin responses and, coupled with the stoichiometric phosphorylation of calmodulin in rat hepatocytes, suggest that it may be relevant in intact cells.

The Solution Structures of Calmodulin and its Complexes with Synthetic Peptides Based on Target Enzyme Binding Domains

Small-angle X-ray and neutron scattering experiments have given important information on the solution structures of calmodulin and its complexes with synthetic peptides used to model target enzyme interactions. In combination with crystallographic data, site directed mutagenesis and various spectroscopic studies, these experiments have contributed to our understanding of the solution structure of calmodulin in different functional states. We have gained important insights into the conformational flexibility in calmodulin that appears to be crucial to its regulatory functions. Specifically, flexibility in the interconnecting helix region of calmodulin has been shown to play a critical role in facilitating calmodulin's binding to a wide variety of target enzymes whose activities are thus regulated. This review will focus mainly on the contributions small-angle scattering has made to our understanding of the

solution structure of calmodulin in the context of other studies, with particular regard to circular dichroism and Fourier transform infrared studies that complement the small-angle scattering data.

Target Sequence Recognition by the Calmodulin Superfamily: Implications from Light Chain Binding to the Regulatory Domain of Scallop Myosin

Some of the rules for how members of the calmodulin (CaM) superfamily bind to target peptides are revealed by the crystal structure of the regulatory domain of scallop myosin. The structure shows that the IQ motif of the heavy chain in this invertebrate myosin imposes constraints on both the positioning and conformation of the individual lobes of the light chains. In contrast, analysis of the contact residues in the targets bound by Ca^{2+}-CaM reveals how the structure of CaM accommodates a broader range of sequences consonant with this protein's functional diversity.

The Calmodulin-binding Domain of the Inducible (Macrophage) Nitric Oxide Synthase

It was thus decided to investigate if synthetic peptides corresponding to the sequence of the macrophage NO-synthase, matching the calmodulin-binding domain of the rat brain enzyme, had the ability to bind calmodulin. The results have shown that this was indeed the case. In keeping with the findings on the intact enzyme, the binding affinity of the macrophage enzyme was found to be much higher than that of the brain synthase. Although optimal binding affinity was observed in the presence of CA^{2+}, calmodulin bound to the peptides with reasonable affinity also in its absence.

PEST Sequences in Calmodulin-binding Proteins

In this report, we discuss the functions of PEST sequences in calmodulin-binding proteins and assess the correlation between calmodulin-binding proteins and PEST sequences.

Overexpression of Ca^{2+}/Calmodulin-Dependent Protein Kinase II Inhibits Neurite Outgrowth of PC12 Cells

These results suggest that CaM kinase II is involved in the modulation of the neurite outgrowth induced by activation of the cyclic AMP system.

The Influence of Temperature During Electric- and Magnetic-Field-Induced Alteration of Calcium-Ion Release from In Vitro Brain Tissue

These findings may reconcile the apparent disagreement in the direction of a field-induced response, and they may explain why experimental outcomes have been difficult to confirm in some laboratories. Of greater importance, the findings may also provide insight into the mechanism of the field-induced phenomonon.

Calcium Signaling in Lymphocytes and ELF Fields

These findings provide evidence that the cell plasma membrane and the calcium channel therein, in contrast to internal cellular structures, is involved in this field interaction. A cell-surface interaction site is mechanistically consistent with an electric field metric since the induced electric field in the media does not significantly penetrate the plasma cell membrane to act on internal cell structures. Preliminary results of this work have been presented in abstract form [11].

Purification and Characterization of Smooth Muscle Myosin Light Chain Kinase

Employing a number of proteolytic inhibitors we report in this paper a procedure for isolating milligram quantities of a homogeneous smooth muscle myosin light chain kinase with a molecular weight of 130,000. Data are presented on the physical, chemical, and kinetic properties of this enzyme in the presence and absence of bound calmodulin.

Smooth Muscle Myosin Light Chain Kinase

In the absence of bound calmodulin, myosin light chain kinase of turkey gizzard is phosphorylated at two sides (A and B). In the presence of bound calmodulin, the kinase is phosphorylated only at site A.

Depolarization Decreases the $[Ca^{2+}]_i$ Sensitivity of Myosin Light-Chain Kinase in Arterial Smooth Muscle: Comparison of Aequorin and Fura 2 $[Ca^{2+}]_i$ Estimates

These data suggest that PKA is not responsible for phosphorylation of MLCK in intact tissues. Potentially, depolarization-induced large increases in $[Ca^{2+}]_i$ could activate Ca^{2+}-calmodulin-dependent protein kinase II and phosphorylate myosin kinase at site A, resulting in desensitization of the myosin phosphorylation system. Our second goal was to evaluate whether the $[Ca^{2+}]_i$ sensitivity of myosin light-chain kinase is altered during stimulation with histamine or depolarization.

Spatial Requirements for Location of Basic Residues in Peptide Substrates for Smooth Muscle Myosin Light Chain Kinase

In this paper, we report the spatial requirements of the enzyme for these basic residues and the contribution of Arg-16.

Role of Myosin Light Chains

All conventional myosin IIs, whether isolated from skeletal, smooth, or invertebrate muscle sources, have two heads attached to an extended 16 nm α-helical coiled-coil tail. The head can be divided into a globular motor domain of ≈770 amino acids that contains the catalytic and actin binding sites, and a neck region of ≈70 amino acids which binds one essential and one regulatory light chain (ELC and RLC).

The Role of the Skeletal Muscle Myosin Light Chains N-terminal Fragments

The myosin regulatory and essential light chains in skeletal muscle do not play a role as significant as in scallop or smooth muscle, however, there are some data suggesting that the skeletal myosin light chains and their N-terminal parts may have a modulatory function in the interaction of actin with myosin heads. In this paper four conformational states of the myosin head with respect to the regulatory light chain bound cation (magnesium or calcium) and phosphorylation are proposed. Communication between regulatory and essential light chains and putative binding of the N-terminus of A1 essential light chain to actin is discussed.

Calmodulin: An Introduction to Biochemical Aspects

The role of calmodulin in general is to detect the Ca^{2+} concentration transients that occur during normal cell function and to then reflect the ambient Ca^{2+} concentration via regulation of various and multiple processes.

The Role of Calcium Ions in Electrically-Stimulated Neurite Formation <u>In Vitro</u>

In this chapter the role of endogenous and applied electric fields in growth and regeneration of nerve tissue are explored. Although nerve tissue uses electricity for communication with and between target tissue, imposition of external electric fields by applying direct current or using Helmholtz coils to induce current have been shown to affect various processes.

Cation Binding to Calmodulin and Relation to Function

This paper deals more specifically with the mode of action of CaM. Although in the last decade our understanding of its action has considerably increased, some enigmas remain and, unfortunately, some basic and long-lasting controversies on its mode of action have not been solved yet. In this paper, two of these are examined: (a) cation binding to CaM, and (b) the thermodynamics of the interaction of CaM with its targets.

The Calcium Pump of the Plasma Membrane: Recent Studies on the Purified Enzyme and on its Proteolytic Fragments, with Particular Attention to the Calmodulin Binding Domain

First, a comparison will be made of the characteristics of the activations by acidic phospholipids and controlled trypsinolysis. Then, the pattern of trypsin proteolysis will be described in some detail, with particular attention to the reconstitution of fragments in liposomes and to the effects of trypsin on the calmodulin binding domain of the molecule. Finally, the effects of chymotrypsin and calpain on the binding of calmodulin to the ATPase and on the organization of the domain that interacts with the activator will be discussed.

A Novel Ca^{2+}-binding Protein Regucalcin and Calcium Inhibition: Regulatory Role in Liver Cell Function

Here, we offer findings made in our laboratory concerning calcium inhibition by regucalcin in liver cells: the physicochemical properties of this protein, inhibition of Ca^{2+} action on the activity of many enzymes, inhibition of Ca^{2+} signaling, its regulatory role in Ca^{2+} homeostasis of liver cells, and the mechanism of action of regucalcin. In many cases, calmodulin and protein kinase C amplify Ca^{2+} action in cells, but this action is reversed by the novel Ca^{2+}-binding protein regucalcin.

Inhibitory and Excitatory Role of Ca^{2+} at Neuromuscular Synapse: The Discovery, Properties, and Role of nACh-RAMIC

External Ca^{2+} may act as a modulator of nAChR channel activity and accelerate desensitization of the receptor. We also observed first noncontractile and contractile Ca^{2+} mobilization, and investigated the direct linkage to nAChR by measuring intracellular Ca^{2+} release.

Molecular Biophysics of Specificity and Function in Enzymes, Receptors and Calcium Binding Proteins

The presentation encompasses the study of a variety of classes of molecules and of the mechanisms in which they function, ranging from the identification of structural and electronic details in a newly proposed mechanism for the enzymatic activity in superoxide dismutase, to structure-function relations and the mechanistic basis for ion selectivity in calcium-binding proteins. In addition, we describe our studies on receptors for the neurotransmitters serotonin and histamine as an illustration of the approach that has served in the elucidation of the chemical basis for ligand recognition and receptor activation processes in neurotransmitter receptor proteins. Inferences from these studies have led to a heuristic scheme for the design of selective ligands with agonist and antagonist properties.

Structure and Function of Calcium-Binding Proteins

The large calcium gradient across the plasma membrane creates different environments for intra- and extracellular calcium-binding proteins. The latter are continuously surrounded by 10^{-3} *M* Ca^{2+}, which promotes activation or stabilization of certain proteases, nucleases, or lipases. Other proteins, such as those involved in blood clotting, contain polyelectrolyte regions that are composed of carbozyglutamic or phosphoserine moieties that allow them to interact with Ca^{2+}. In contrast, intracellular calcium-binding proteins, such as calmodulin and troponin C, the trigger proteins for muscle contractions, need to respond to an increase in Ca^{2+} from 10^{-7} to 10^{-6} *M* during cell activation. Evidence is presented that the pairwise arrangement of characteristic helix-loop-helix calcium-binding sites can result in the positive cooperative binding of Ca^{2+}. This can be further promoted by the binding of ligands, drugs, or target proteins. Several drug binding sites on calmodulin are allosterically related and their localization on the unusual dumbbell structure of this molecule will be discussed.

Myosin Structure and Function

In this chapter we will describe the general features of the molecule that is the molecular motor of vertebrate smooth muscle cells.

Myosin Regulation and Asssembly

This review describes some structural elements that are necessary to activate myosin's motor properties and to stabilize the folded monomeric conformation.

Myosin Light Chains

In this chapter we review the structure-function relationship of vertebrate smooth muscle myosin LCs. The LCs are the protein cofactors of the smooth muscle contractile machinery, and we will focus on the experiments that investigate the mechanisms of their functional role. We also describe basic methods for the study of the LCs, with special emphasis on the procedures needed for the characterization of the phosphorylated LC20.

Calcium Binding Proteins

The purpose of this chapter is to describe the calcium binding properties of these calcium binding proteins with special emphasis on CaT. With CaT we will demonstrate how it can regulate CaD in a calcium-dependent manner. In this context, the effect of CaT on the CaD-myosin and the CaD-actin interactions in the presence of Ca^{2+} will be discussed.

Myosin Light Chain Kinase

This chapter will focus on the biochemical properties of MLCK in relation to its activation in smooth muscle and to recent insights into the molecular structure of the catalytic core and its regulation by an autoinhibitory region and calmodulin binding domain.

Effects of ELF Fields on Calcium-Ion Efflux from Brain Tissue

We report here that 16-Hz sinusoidal fields in the absence of a carrier wave can alter the efflux rate of calcium ions. The results show a frequency-dependent, field-induced enhancement of calcium-ion efflux within the ranges 5 to 7.5 V/m and 35 to 50 V/m (peak-to-peak incident field in air) with no enhancement within the ranges 1 to 2, 10 to 30, and 60 to 70 V/m.

Comparison of Contractile Properties between Developing and Regenerating Soleus Muscle: Influence of External Calcium Concentration upon the Contractility

In newborn rat skeletal extensor digitorum longus (EDL) muscle, it has been found that an influx of calcium from the extracellular medium is necessary for contraction, in contrast to the situation observed in adult EDL muscle. The aim of the present study was to determine the influence of the extracellular calcium concentration ([Ca]o) upon the contractile responses elicited in developing as well as in regenerating (notexin-injected) soleus (SOL) muscle. A morphological study was performed to follow the steps of postnatal development and regeneration in SOL muscle. In nominally calcium-free solution, the amplitudes of the twitch and tetanic tensions were greatly reduced in 1-14- day-old developing SOL muscles, as well as in notexin-injected SOL muscles. With longer times after birth, twitch and tetanic tensions of SO muscle were less affected by the absence of calcium. This contrasts with notexin-injected SOL muscle in which the amplitudes of the contractions remained strongly dependent on [Ca]o. The present finding suggests that some functional characteristics are different in regenerating muscle fibers and may be of interest in the evaluation of the contractile properties of muscles in which injections of genetically engineered or not autologous myoblasts or viral vector have been performed.

Intracellular Calcium and Force in Single Mouse Muscle Fibres Following Repeated Contractions with Stretch

In this study we describe changes in muscle properties following the isometric series as 'fatigue' and changes in muscle properties following the stretch series which are greater than those following the isometric series are described as 'stretch induced.'

From Muscle Properties to Human Performance, Using Magnetic Resonance

Our goal is to show how muscle properties can be used to understand the exercise performance limitations of the elderly. We show that magnetic resonance (MR) imaging and spectroscopy are useful for noninvasively characterizing the structural and energetic properties of muscle in vivo. Determination of muscle volume and cross-sectional area is easily and rapidly accomplished by applying quantitative morphometric methods to MR images. New MR spectroscopic techniques provide a noninvasive "biopsy" of the oxidative, glycolytic, and contractile capacities of muscle fibers. We show how the structural and energetic properties measured by MR can be used to define the functional capacity of muscle and the contribution of this capacity to the performance of the whole body (e.g., Vo_2max). Finally, we relate these laboratory measures of muscle properties and performance to activities meaningful to the functioning of the elderly in everyday life, such as sustained walking and stair climbing.

Fatigue and Heat Production in Repeated Contractions of Mouse Skeletal Muscle

The purpose of this study was to examine fatigue related to cellular energy use. The combination of high duty cycles and the high stimulation frequencies required to elicit maximum tetanic force may result in failure of sarcolemmal excitation (Nassar-Gentina, Passoneau & Rappoport, 1981.)

Origin of Muscle Action Potentials Evoked by Transcranial Magnetic Stimulation in Cats

This finding strongly suggests that the generator of the MEPs resides in the brainstem, mainly at the vestibular nuclei complex.

Actin and the Actomyosin Interface: A Review

The result is an actin-centered view of the loci on actin which are probably involved in its interaction with the myosin 'head.' From these multiple contacts we speculate on the sequence of steps between the initial weak-binding state of S-1 to the actin filament through to the stable strong-binding state seen in the absence of free Mg-ATP, i.e., the rigor state.

Ca Reversal and Ca Relaxation – Ca Inhibition of Ca-independent Contraction of Smooth Muscle

Here, we limit ourselves to Ca-free contraction elicited by oxytocin in the uterine longitudinal smooth muscle of the estrogen-dominated rat, which has been otherwise specially mentioned.

Determination of Resting Free Calcium in Barnacle Muscle Using Modified Aequorins, Buffered Calcium Injections, and Simultaneous Image-intensified Video Microscopy

Using these methods, we estimated the free calcium level in the lateral depressor fibres of freshly dredged barnacles to by 279 ± 36 nM (±SD), 399 ± 42 nM, or 352 ± 45 nM for the linear, 2.2 and 2.5 powers respectively under the conditions of hch-aequorin and BAPTA buffers (using a K'_{Ca} for BAPTA of 3.0 x 10^6 M^{-1} for our conditions.) Recombinant-aequorin gave essentially the same result while EGTA buffers yielded a somewhat higher value but because of influences of pH on the K'_{Ca} for EGTA (taken as 6.7 x 10^6 M^{-1} for our conditions) was considered less reliable. Minor changes in $[Mg^{2+}]$ upon buffer injection can lead to underestimates of the true resting $[Ca^{2+}]$ by at most 10%. Thus, we estimate the resting free calcium in barnacle muscle fibres to be 300-380 nM.

Calmodulin is Essential from Smooth Muscle Contraction

It is concluded that CaM is essential for Ca^{2+} regulation of skinned smooth muscle. Its action is compatible with stimulation of MLCK to phoshorylate myosin light chain.

Electrical and Mechanical Responses Produced by Nerve Stimulation in Detrusor Smooth Muscle of the Guinea-Pig

It is concluded that nerve stimulation releases acetylcholine and ATP, and the former produces contraction without change in the membrane potential, while the latter generates the e.j.p. which triggers an action potential and thus elicits contractions.

Effects of Actin and Calcium Ion on Chymotryptic Digestion of Skeletal Myosin and Their Implications to the Function of Light Chains

These results are explained in terms of localized changes within the Nbs_2 light chains and subfragment 1. Subunit interactions in the myosin head lead to a Ca^{2+}-induced reduction in the affinity of heavy meromyosin for actin in the presence of MgATP. The resulting Ca^{2+} inhibition of the actin-activated ATPase of myosin can be detected at high salt concentrations (75 mM KCl).

Myosin II Function in Non-muscle Cells

Amongst the remarkable variety of motility that cells display, cytokinesis (cell division) is particularly striking. Dramatic changes in cell shape occur before, during and after cytokinesis. Myosin II is implicated in the 'rounding up' of cells prior to cytokinesis, and is essential in the formation of the contractile cleavage furrow during cytokinesis. Now it appears that myosin II plays a role in all stages of cytokinesis, as a recent report suggests that myosin II drives post-mitotic cell spreading. A similar type of motile mechanism operating in cell spreading may occur in other cell types in other situations.

Myosin Light Chain Phosphorylation During Contraction of Chicken Fast and Slow Skeletal Muscles

In this paper, we show that maximal light chain phosphorylation in the fast posterior latissimus dorsi (PLD)[1] muscle of chicken closely follows the maximal isometric tension development. Light chain phosphorylation in the slow anterior latissimus dorsi (ALD) proceeds at a much slower rate than in the fast PLD. In both types of muscles light chain dephosphorylation lags far behind muscle relaxation.

Indirect Coupling of Phosphate Release to *de novo* Tension Generation During Muscle Contraction

We conclude that tension generation occurs in the absence of chemical change as the result of an entropy-driven transition between strongly bound crossbridges in the actomyosin-ADP state. The mechanism resembles the operation of a clock, with phosphate release providing the energy to tension the spring, and the irreversible step functions as the escapement mechanism, which is followed in turn by tension generation as the movement of the hands.

Effect of Noxious Stimulation on Sympathetic Vasoconstrictor Outflow to Human Muscles

It is concluded that sustained noxious stimulation in awake humans evokes a generalized MSA increase; the activity is still under baroreflex control, but the inhibitory level is reset. Both spinal and brainstem reflexes may contribute; a defence reaction is an unlikely explanation. It is suggested that the number of afferent C fibres activated by electrical stimulation of digital nerves was insufficient to induce any marked MSA response.

Intracellular Calcium, Myosin Light Chain Phosphorylation, and Contractile Force in Experimental Cerebral Vasospasm

The increased intracellular calcium concentration and increased percent myosin light chain phosphorylation in vasospastic segments implicate a role for the Ca^{2+}-dependent pathway of smooth muscle cell contraction in vasospasm.

Different Mechanisms for Ca^{2+} Dissociation from Complexes of Calmodulin with Nitric Oxide Synthase or Myosin Light Chain Kinase

We have determined the stoichiometry and rate constants for the dissociation of Ca^{2+} ion from calmodulin (CaM) complexed with rabbit skeletal muscle myosin light chain kinase (skMLCK), rat brain nitric oxide synthase (nNOS) or with the respective peptides (skPEP and nPEP) representing the CaM-binding domains in these enzymes.

Requirements for Calcium and Calmodulin in the Calmodulin Kinase Activation Cascade

Ca^{2+}/calmodulin-dependent protein kinase IV (CaM-K IV)[1] (1-3), a member of the CaM-kinase family, mediates Ca^{2+}-dependent transcriptional activation through phosphorylation of transcription factors such as cAMP response element binding protein (4-7) and serum response factor (8,9).

Movement and Force Produced by a Single Myosin Head

Our analysis accounts for the broad distribution of displacement amplitudes observed, and indicates that the underlying movement (working stroke) produced by a single acto-S1 interaction is ~4nm, considerably shorter than previous estimates but consistent with structural data. We measure the average force generated by S1 or HMM to be at least 1.7 pN under isometric conditions.

Calcium/Calmodulin-dependent Protein Kinase II Downregulates Both Calcineurin and Protein Kinase C-mediated Pathways for Cytokine Gene Transcription in Human T Cells

These results suggest that CaM-K II may exert negative influences on cytokine gene transcription in human T cells, and provide preliminary evidence for negative cross-talk with the calcineurin- and PKC-dependent signaling systems.

Calcium-Binding Proteins in Health and Disease

The structure of calmodulin was originally reported at a resolution of 3.0 Å. It is currently being refined at 2.2 Å resolution, and although the refinement is still incomplete, we can now report in greater detail certain secondary structural features of calmodulin.

Regulation of Myosin Light Chain Kinase by Reversible Phosphorylation and Calcium-Calmodulin

The contractile proteins actin and myosin are present in most, if not all, eukaryotic cells. Their role in muscle contraction has been well-documented in all types of muscle cells: skeletal, cardiac, and smooth. Their function in nonmuscle cells is still obscure, but they are thought to play a role in cell migration, cytokinesis, and possibly in cell division. In addition they are also through to function in specialized cell processes, such as phagocytosis in macrophages and clot retraction in blood platelets.

Effect of Multiple Phosphorylations on Movement of Smooth Muscle and Cytoplasmic Myosin

In general, smooth muscle myosin-coated beads moved at about 5-10% of the velocity of skeletal muscle myosin-coated beads and about 5 times as fast as the cytoplasmic myosin-coated beads. We also observed that the velocity of skeletal muscle myosin-coated beads was not effected by phosphorylation of the light chains by skeletal muscle MLC kinase (data not shown).

Calcium/Calmodulin-Dependent Protein Kinases

In the first section, we will describe the molecular characteristics of both types of these CaM kinases. Their characteristics are summarized in Table I.

GPI-anchored Influenza Hemagglutinin Induces Hemifusion to Both Red Blood Cell and Planar Bilayer Membranes

Under fusogenic conditions, fluorescent dye redistributed from the outer monolayer leaflet of red blood cells (RBCs) to cells expressing glycophosphatidylinositol-anchored influenza virus hemagglutinin (GPI-HA) without transfer of aqueous dye. This suggests that hemifusion, but not full fusion, occurred (Kemble, G.W., T. Danieli, and J.M. White. 1994. Cell. 76:383-391). We extended the evidence for hemifusion by labeling the inner monolayer leaflets of RBCs with FM-64 and observing that these inner leaflets did not become continuous with GPI-HA-expressing cells. The region of hemifusion-separated aqueous contents, the hemifusion diaphragm, appeared to be extended and was long-lived. But when RBCs hemifused to GPI-HA-expressing cells were osmotically swollen, some diaphragms were disrupted, and spread of both inner leaflet and aqueous dyes was observed. This was characteristic of full fusion: inner leaflet and aqueous probes spread to cells expressing wild-type HA (wt-HA). By simultaneous video fluorescence microscopy and time-resolved electrical admittance measurements, we rigorously demonstrated that GPI-HA-expressing cells hemifuse to planar bilayer membranes: lipid continuity was established without formation of fusion pores. The hemifusion area became large. In contrast, for cells expressing wt-HA, before lipid dye spread, fusion pores were always observed, establishing that full fusion occurred. We present an elastic coupling model in which the ectodomain of wt-HA induces hemifusion and the transmembrane domain, absent in the GPI-KA-expressing cells, mediates full fusion.

Calcium Control of Muscle Phosphorylase Kinase through the Combined Action of Calmodulin and Troponin

1. Discovery of the calcium control of phosphorylase kinase; 2. Identification of the δ subunit of phosphorylase kinase as calmodulin; 4. Phosphorylase kinase binds a second molecule of calmodulin, termed the δ' subunit, which produces additional activation of the enzyme; 5. Interaction of phosphorylase kinase with the δ and δ' subunits, and identification of the calmodulin binding subunits; 6. Troponin C, the troponin Complex, and artificial thin filaments can substitute for the δ' subunit in the activation of phosphorylase kinase; 7. Regulation of the different forms of phosphorylase kinase by Ca^{2+}, calmodulin, and troponin; 8. Proportion of the calmodulin in skeletal muscle that is bound to phosphorylas kinase; 9. Activation of phosphorylase kinase b by troponin may be the key event in coupling glycogenolysis and contraction; 10. Regulation of phosphorylase kinase a by Ca^{2+}; 11. Summary – The current evidence suggests that the regulation of phosphorylase kinase by Ca^{2+} *in vivo* is achieved through the interaction of this divalent cation with calmodulin (the δ subunit) and troponin C, and that the relative importance of these two calcium binding proteins depends on the state of phosphorylation of the enzyme. In the low-activity dephosphorylated b form, increasing Ca^{2+} from 0.1 μM to concentrations in the μM range produces a 5-10-fold activation through the binding of Ca^{2+} to the δ subunit, and a further 15-25-fold activation through the binding of Ca^{2+} to troponin C. Troponin C rather than the δ subunit is therefore the dominant calcium dependent regulator of the b form, providing an attractive mechanism for coupling glycogenolysis and muscle contraction. On the other hand, the high-activity phoshorylated a form is only activated very slightly by troponin. The δ subunit is therefore the dominant calcium dependent regulator of the hormonally activated state of the enzyme.

Calcium-Dependent Protein Kinases and Calmodulin Antagonists

This article focuses on the basic properties of calcium-activated protein kinases as potential targets for calcium antagonists. Due to the limited amount of space available, reference will be given mostly to new articles not covered by recent reviews.

Presence and Possible Involvement of Ca/Calmodulin-Dependent Protein Kinases in Insulin Release from the Rat Pancreatic β Cell

These results suggest that Ca/CaM kinase II and MLCK may participate in the control of insulin release.

Regulation of Ca^{2+}/Calmodulin-Dependent Protein Kinase II by Autophosphory lation/Dephosphorylation

The following studies have examined the effects of autophosphorylation on soluble and postsynaptic density-associated forms of CaM-KII from brain.

Activation of Skeletal Muscle Myosin Light Chain Kinase by Calcium(2+) and Calmodulin

A detailed analysis of skeletal muscle myosin light chain kinase activation was undertaken in order to determine the stoichiometries and equilibrium constants of Ca^{2+}, calmodulin, and enzyme catalytic subunit in the activation process. The analysis indicates that activation is a sequential, fully reversible process requiring both Ca^{2+} and calmodulin.

Calcium and Calmodulin

A description of the value of calcium and calmodulin is given in terms of their dynamics and structures. It is insisted that a full appreciation of their value demands recognition of the time domain, motion, in evolution in concert with structure. Only in this way can homeostasis of resting states as well as triggering, assisted by calcium/calmodulin, be described. The best analogy is with a regulatory electrical control such as household central heating system where calcium carries the current and calmodulin triggers the heating (cellular work) and the pumps (cellular pumps). There is full feed-back control.

Calcium-Calmodulin Modulation of the Olfactory Cyclic Nucleotide-Gated Cation Channel

The data reveal a control mechanism that resembles those underlying the regulation of enzymes by calmodulin. The results also point to the amino-terminal part of the olfactory channel as an element for gating, which may have general significance in the operation of ion channels with similar overall structures.

Vascular Aldosterone. Biosynthesis and a Link to Angiotensin II-Induced Hypertrophy of Vascular Smooth Muscle Cells

Analysis of human MR gene expression demonstrated that both the vascular EC and VSM expressed the published MR mRNA and a smaller, apparently alternatively spliced, MR mRNA which was predicted its structure to be functional.

Regulation of Embryonic Smooth Muscle Myosin by Myosin Light Chain Kinase and by Protein Kinase C

The presence of developmentally regulated myosin isoforms in tissues is well established (1-9). Cardiac myosin has been studied extensively, and three different isoforms have been identified (4). These myosin isoforms differ with respect to (a) their mobilities nondenaturing polyacrylamide gels (3), (b) subunit composition (3) and (c) actomyosin ATPase activities (4). Moreover, specific cardiac myosin isoforms are expressed following volume overload of the heart and change depending upon thyroid hormone levels (5). Similarly, specific myosin isoforms are expressed in skeletal muscle, and the myosin isoform correlates with both the type of innervation and the sequence of electrical stimulation (6).

Myosin Light Chain Kinase Binding to Plastic

Inclusion of detergent, Tween-80, in the enzyme dilution and assay buffers prevented the loss of enzyme due to adsorption onto plastic surfaces and allowed an accurate measure of its specific activity.

Modulator Protein as a Component of the Myosin Light Chain Kinase from Chicken Gizzard

In this communication it is demonstrated that the 17 000 component is the modulator protein. This conclusion is based on: (1) the identical behavior of the 17 000 kinase component and modulator protein in assays of actomyosin Mg^{2+}-ATPase activity, phosphorylation of myosin, and phosphodiesterase activity, and, (2) the similarity of the 17 000 kinase component and the modulator protein with respect to amino acid composition, absorption spectrum, and electrophoresis in urea-polyacrylamide gels. It is shown also that the modulator protein from smooth muscle and troponin C are distinct proteins.

Organization of Myosin Light Chain Kinase from Rabbit Skeletal Muscle

In this article we will focus exclusively on the isozyme of MLCK which is obtained from rabbit skeletal muscle. Special emphasis will be placed upon relating recent insights concerning the structure of MLCK to the organization of its functional domains and its regulation by Ca/CaM.

Isotope-Filtered 2D NMR of a Protein-Peptide Complex: Study of a Skeletal Muscle Myosin Light Chain Kinase Fragment Bound to Calmodulin

An NMR approach is demonstrated for elucidating the conformation of a peptide when tightly bound to a protein. A 26-residue peptide derived from rabbit skeletal muscle myosin light chain kinase, comprising the binding site for calmodulin, was complexed with uniformly (>95%) ^{15}N- and ^{13}C-enriched calmodulin. Improved isotope-filtered two-dimensional NMR techniques were developed for suppressing NMR calmodulin signals. NOE patterns indicate that residues Arg-3 through Ser-21 of the bound peptide form an α-helix.

New Experimental Technique for Detecting the Effect of Low-Frequency Electric Fields on Enzyme Structure

The results indicate that the technique is sensitive to conformational changes that otherwise may be impossible to detect. However, exposure to electric currents under the experimental conditions described herein showed no effects of the currents.

Contraction due to Microtubule Disruption is Associated with Increased Phosphorylation of Myosin Regulatory Light Chain

Our results suggest that phosphorylation of LC_{20} is a common mechanism for the contractions stimulated both by microtubule poisons and receptor-mediated agonists. The modulation of myosin activity by alterations in microtubule assembly may coordinate the physiological functions of these cytoskeletal components.

Effects of Myosin Light Chain Kinase Inhibitors on Delayed Rectifier Potassium Current in Bullfrog Sympathetic Neurons

Results suggest that phosphorylation of myosin may modulate kinetics for the inactivation of I_K.

A Ca^{2+}- and Modulator-Dependent Myosin Light Chain Kinase from Non-Muscle Cells

It is suggested that the role of the myosin light chain kinase is similar in both muscle and non-muscle cells and serves to activate the contractile apparatus, via the phosphorylation of myosin, in response to an increase in the intracellular free Ca^{2+} concentration.

Composition of the Myosin Light Chain Kinase from Chicken Gizzard

The Ca^{2+}-dependent protein kinase (ATP: myosin light chain phosphotransferase) from chicken gizzard smooth muscle requires two proteins for enzymatic activity. These have approximate molecular weights of 105,000 and 17,000 daltons. The isolation procedure for each component is described. Neither component alone markedly alters either the actin-moderated ATPase activity or the phosphorylation of myosin. Activation of ATPase activity by a combination of the two components occurred only in the presence of Ca^{2+} and was always accompanied by the phosphorylation of myosin. The simultaneous activation of ATPase activity and myosin phosphorylation establishes a direct correlation between the two events.

Myosin Light Chain Kinase in Skinned Fibers

The work reviewed in this chapter is primarily concerned with the use of skinned muscle fibers to elucidate the physiological role of myosin light chain kinase in the regulation of muscle contraction.

Spatial Requirements for Location of Basic Residues in Peptide Substrates for Smooth Muscle Myosin Light Chain Kinase

These results support the concept that both the presence and location of basic residues play an essential role in the substrate specificity of the smooth muscle myosin light chain kinase.

Dephosphorylation of a 30-kDa Protein of Fowl Spermatozoa by the Addition of Myosin Light Chain Kinase Substrate Peptide Inhibits the Flagellar Motility

These results suggest that the 30 kDa protein is identified as a substrate for MLCK or a MLCK-like protein in fowl spermatozoa and that phosphorylation-dephosphorylation of this protein is involved in the regulation of flagellar movement at 30°C.

The Human Myosin Light Chain Kinase (MLCK) from Hippocampus: Cloning, Sequencing, Expression, and Localization to 3qcen-q21

We show that within the protein sequence, a motif of 28 or 24 residues is repeated five times, the second repeat ending with the putative methionine start codon.

Increased Phosphorylation of Ca^{2+}/Calmodulin-dependent Protein Kinase II and Its Endogenous Substrates in the Induction of Long Term Potentiation

These results indicate that activation of CaM kinase II is involved in the induction of synaptic potentiation in both the postsynaptic and presynaptic regions.

Structure of the Pseudosubstrate Recognition Site of Chicken Smooth Muscle Myosin Light Chain Kinase

The structure of the chicken smooth muscle myosin light chain kinase pseudosubstrate sequence MLCK(774-807)amide was studies using two-dimensional proton NMR spectroscopy.

Differential Targeting of Protein Kinase C and CaM Kinase II Signalings to Vimentin

These results indicate that the intracellular targeting of C kinase and CaM kinase II signalings to vimentin is regulated separately, under physiological conditions.

Myosin II Filament Assemblies in the Active Lamella of Fibroblasts: Their Morphogenesis and Role in the Formation of Actin Filament Bundles

We propose that zig-zag assemblies of myosin II filaments induce the formation of actin bundles by pulling on an actin filament network and that co-alignment of actin and myosin filaments proceeds via folding of myosin II filament assemblies in an accordion-like fashion.

Both the Amino and Carboxyl Termini of Dictyostelium Myosin Essential Light Chain are Required for Binding to Myosin Heavy Chain

Since normal myosin function requires association of the ELC, we set out to map the domains of the ELC that are critical for ELC-MHC interaction.

Regulation and Kinetics of the Actin-Myosin-ATP Interaction[1,2]

The purpose of this article is, first, to review the essential features of the three major regulatory systems of muscle contraction: the binding of Ca^{2+} to troponin-tropomyosin, the phosphorylation of myosin by a Ca^{2+}-dependent kinase, and the direct binding of Ca^{2+} to myosin; and second, to review the kinetics of the actin-myosin-ATP interaction; then, to consider possible biochemical mechanisms by which the regulatory systems could turn muscle contraction on and off.

Effects of ELF (1-120 Hz) and Modulated (50 Hz) RF Fields on the Efflux of Calcium Ions from Brain Tissue In Vitro

We now demonstrate that although there is no enhanced efflux associated with a 42-Hz field at 30, 40, 50, or 60 V_{p-p}/m, a 45-Hz field causes enhanced efflux in an intensity range around 40 V_{p-p}/m that is essentially identical to the response observed for 16-Hz fields.

A Role for the Magnetic Field in the Radiation-Induced Efflux of Calcium Ions from Brain Tissue In Vitro

The results identify the AC magnetic component as essential for the efflux enhancements observed in our laboratory. The demonstrated importance of the AC magnetic component led to an additional examination of a possible role for the local geomagnetic field (LGF) in this phenomenon.

Radiofrequency Radiation-Induced Calcium Ion Efflux Enhancement from Human and Other Neuroblastoma Cells in Culture

To test the generality of radiofrequency radiation-induced changes in $^{45}Ca^{2+}$ efflux from avian and feline brain tissues, human neuroblastoma cells were exposed to electromagnetic radiation at 147 MHz, amplitude-modulated (AM) at 16 Hz, at specific absorption rates (SAR) of 0.1, 0.05, 0.01, 0.005, 0.001, and 0.0005 W/kg.

Time-varying and Static Magnetic Fields Act in Combination to Alter Calcium Signal Transduction in the Lymphocyte

We have tested the hypothesis that extremely low frequency (ELF) time-varying magnetic fields act in combination with static magnetic fields to alter calcium signaling in the lymphocyte. Results indicate that

Interaction Between Calmodulin and Target Proteins

This result suggests that mastoparan can be used as a model compound of MLCK. The measurements of Ca^{2+} binding to calmodulin by equilibrium dialysis or flow dialysis in the presence of MLCK (target enzymes) were difficult experiments because of their high molecular weights. The detailed study of ^1H-NMR of calmodulin in the presence of MLCK was almost impossible. Therefore, a small molecular weight model compound is useful for the extensive investigations of the effect of MLCK to calmodulin.

Calmodulin

Like cAMP, the calmodulin·Ca^{2+} complex is a universal regulator that is not tissue specific, not species specific, extremely well-conserved during evolution, and affects a large number of cellular functions. In each of these respects calmodulin is very different from the other Ca^{2+}-binding proteins (troponin C, parvalbumin, S-100, and intestinal Ca^{2+}-binding protein) which are also believed to have evolved from a common ancestor to perform specific functions (7,8).

Protein Engineering and NMR Studies of Calmodulin

In this contribution, we discuss the features of CaM, which allow it to be rather promiscuous, and bind effectively to all these distinct domains. In particular, we describe the role of the methionine-rich hydrophobic surfaces of the protein in providing a malleable and sticky surface for binding many hydrophobic peptides.

High Molecular Weight Calmodulin-Binding Protein is Phosphorylated by Calmodulin-Dependent Protein Kinase V1 from Bovine Cardiac Muscle

The phosphorylation resulted in the maximal incorporation of 1 mol of phosphate/mol of the HMW CaMBP. The distinct substrate specificity of this protein kinase indicates that it is not related to the known protein kinases (I, II, III, IV< and V) that have been already characterized, therefore we would like to designate this novel kinase as a CaM-dependent protein kinase V1.

Calmodulin Plays a Pivotal Role in Cellular Regulation

The role of calcium ions (Ca^{2+}) in cell functions is beginning to be unraveled at the molecular level as a result of recent research on calcium-binding proteins and particularly on calmodulin. These proteins interact reversibly with Ca^{2+} to form a protein · Ca^{2+} complex, whose activity is regulated by the cellular flux of Ca^{2+}. Many of the effects of Ca^{2+} appear to be exerted through calmodulin-regulated enzymes.

Interactive Properties of Calmodulin

This review deals more specifically with the cation binding properties of CaM in the absence and presence of its target enzymes. Although our understanding has considerably increased (Kilhoffer *et al.*, 1983; Cox *et al.*, 1984; Forsen *et al.*, 1986) some enigmas remain and, unfortunately, some basic and long-lasting controversies have not yet been solved.

A Non-Selective Cation Current Activated via the Multifunctional Ca^{2+}-Calmodulin-dependent Protein Kinase in Human Epithelial Cells

We have observed that elevation of intracellular calcium in single T_{84} epithelial cells simultaneously activates both a chloride and a non-selective cation current in a transient manner. Our findings indicate that endogenous CaM kinase mediates the activation of not only the chloride current but also the non-selective cation current in these cells. Furthermore, evidence is provided that the calcium-activated single cation channel events we observe in excised membrane patches underlie the macroscopic cation current activated by CaM kinase in intact cells.

Biophysical Studies of Calmodulin

Instead, we will focus our attention on some biophysical studies that have been reported during the last few years and, using these results, attempt to present a more detailed picture of CaM's physical mode of action than was hitherto possible.

Preparation and Properties of the Calmodulin-Binding Domain of Skeletal Muscle Myosin Light Chain Kinase

The purpose of this chapter is to review the procedures used to identify and characterize the calmodulin-binding domain of rabbit skeletal muscle MLCK in the hope that the procedures used for the studies of this enzyme might be readily adapted by other investigators for the studies of other calmodulin-dependent enzymes.

Structure of Calmodulin Refined at 2·2 Å Resolution

The molecule is shaped somewhat like a dumbbell, with an overall length of 65 Å; the two lobes are connected by a seven-turn α-helix. Prominent secondary structural features include seven α-helices, four Ca^{2+}-binding loops, and two short, double-stranded antiparallel beta-sheets between pairs of adjacent Ca^{2+}-binding loops. The four Ca^{2+}-binding domains in calmodulin have a typical EF hand conformation (helix-loop-helix) and are similar to those described in other Ca^{2+}-binding proteins. The X-ray structure determination of calmodulin shows a large hydrophobic cleft in each half of the molecule. These hydrophobic regions probably represent the sites of interaction with many of the pharmacological agents known to bind to calmodulin.

Biopharmacological Assessment of Calmodulin Function: Utility of Calmodulin Antagonist Naphthalenesulfonamide

Our pharmacological studies using calmodulin antagonists have demonstrated that calmodulin is involved in Ca^{2+} regulation of actin-myosin interaction of smooth muscle (8-10).

Activity-Structure Relationship of Calmodulin Antagonists

The role of calmodulin in vascular response was investigated using two series of synthesized naphthalenesulfonamide derivatives. The actions of these compounds as calmodulin antagonists and vascular relaxants were shown to depend both on the chlorination of the naphthaline ring and on the length of the alkyl chain (C_5-C_{10}).

Interaction of Calmodulin and a Calmodulin-Binding Peptide from Myosin Light Chain Kinase: Major Spectral Changes in Both Occur as the Result of Complex Formation

In the absence of Ca^{2+}, the measured ellipticity of the mixture is approximately the sum of the two components. Addition of the peptide to calmodulin causes dramatic changes in the proton NMR spectrum; at a 1:1 molar ration, no evidence of either free peptide or free calmodulin is observed. Moreover, these data demonstrate that a unique species of the M13-calmodulin complex is formed, indicating that the peptide binds to calmodulin in only one way. The many resonances affected by M13 binding include residues in both halves of the calmodulin molecule. The observed CA and NMR effects suggest that secondary and tertiary conformational changes occur both in M13 and in calmodulin upon complex formation. Thus, changes in calmodulin tertiary structure following protein binding may represent an additional step in the presently accepted mechanism for calmodulin-dependent activation of MLCK and other target proteins.

Calmodulin-Binding Domain of Myosin Light Chain Kinase

Small-angle X-ray and neutron scattering data were used to study the solution structure of calmodulin complexed with a synthetic peptide corresponding to residues 577-603 of rabbit skeletal muscle myosin light chain kinase.

Calmodulin and Myosin Light-Chain Kinase of Rabbit Fast Skeletal Muscle

The present paper reports a reinvestigation of the nature of the myosin light-chain kinase system of rabbit skeletal muscle. We conclude that the whole myosin light-chain fraction used as substrate in our earlier studies (Pires & Perry, 1977).

An Interdisciplinary Approach to the Molecular Mechanisms of Calmodulin Action: Comparative Biochemistry, Site-Specific Mutagenesis, and Protein Engineering

We describe in this report one example of how we are utilizing this protein engineering approach in conjunction with studies of selected calmodulin binding proteins and their catalytic activities.

Crystal Structure of Calmodulin

After discussing the structure determination and describing the structure itself, we relate various other studies to the anatomy of calmodulin as seen in the crystal.

Biopharmacological Assessment of Calmodulin Function: Utility of Calmodulin Antagonists

Pharmacological studies using calmodulin antagonists such as phenothiazines and naphthalenesulfonamides suggest that calmodulin may play an important role in platelet function (25), cell proliferation (10), vascular contraction (12), insulin secretion (29), receptor mediated endocytosis (28), and others. We propose that calmodulin antagonists should be defined as agents which bind to calmodulin calcium dependently and inhibit selectively calcium calmodulin dependent enzymes. In this paper, we will describe the character of calmodulin antagonists, the binding sites on calmodulin, the activity-structure relationship of these agents and the useful application of these antagonists to biological functions such as human platelet secretion, vascular smooth muscle contraction and cell proliferation. Particular emphasis is placed on the biological importance of sister compounds which are chlorinated and dechlorinated naphthalenesulfonamide.

Actions of Calmodulin and Cyclic Nucleotides in Vascular Smooth Muscles: Assessments from Drug Actions

In this article, we direct attention to the actions of isoprenaline and nitroglycerine on vascular smooth muscles in relation to the actions of calmodulin and cyclic nucleotides on contractile proteins, Ca accumulation into intracellular storage sites, and Ca extrusion from the muscle cell.

Solution Structure of Calmodulin and its Complex with a Myosin Light Chain Kinase Fragment

The solution structure of Ca^{2+} ligated calmodulin and of its complex with a 26-residue peptide fragment of skeletal muscle myosin light chain kinase (skMLCK) have been investigated by multi-dimensional NMR. In the absence of peptide, the two globular domains of calmodulin adopt the same structure as observed in the crystalline form [2]. The so-called 'central helix' which is observed in the crystalline state is disrupted in solution. ^{15}N relaxation studies show that residues Asp78 through Ser81, located near the middle of this 'central helix', form a very flexible link between the two globular domains. In the presence of skMLCK target peptide, the peptide-protein complex adopts a globular ellipsoidal shape. The helical peptide is located in a hydrophobic channel that goes through the center of the complex and makes an angle of ~45° with the long axis of the ellipsoid.

Calmodulin: An Introduction to Biochemical Aspects

In an attempt to clarify the situation Cheung *et al.* (1978) proposed the term calmodulin to indicate that calcium is involved and also that the protein serves a modulating function.

Calmodulin and Calcium-Binding Proteins: Evolutionary Diversification of Structure and Function

This chapter will focus on the evolution of the family of intracellular Ca^{2+}-binding proteins with respect to their involvement in transduction of the calcium signal or its suppression.

Stimulation of Synthesis of Neurotransmitters by Calmodulin-Dependent Phosphorylation

In summary, the activation of tryptophan hydroxylase by phosphorylating conditions (Kuhn *et al.*, 1980) satisfies all but one of the criteria set forth by Cheung (1980) for identifying CaM-dependent processes. These include: (1) a demonstration that CaM is endogenous to tissue in use, (2) depletion of CaM from tissue alters the activity under study, (3) the CaM effect should be Ca^{2+}-dependent, and (4) the effect should be antagonized by trifluoperazine or other (CaM-binding neuroleptic drugs.

Regulatory and Target-Binding Domains of Calmodulin

The results of Ca^{2+}-binding were compared with ^1H NMR spectrum of calmodulin in the presence of equimolar concentration of mastoparan. We conclude from these results that Ca^{2+}-saturated C-domain bound to target enzyme induces a positive cooperative Ca^{2+} binding to N-domain which works as a regulatory domain.

Microwave Enchancement of Membrane Conductance: Calmodulin Hypothesis

It has been found that 2450 MHz microwave radiation increases membrane conductance in molluscan neurons. Analysis of this effect points to the important role of Ca^{++} in the mechanism of neuron microwave response. However, regulation of many intracellular processes is not a direct Ca^{++} effect, but is mediated through calmodulin, a Ca^{++}-binding multifunctional protein. Furthermore, there is some evidence showing that Ca^{++} regulation of a Ca pump, endoplasmic reticulum Ca^{++} buffering, and Ca^{++}-activated K^+ conductance are mediated via calmodulin. Based on that, calmodulin is hypothesized to be a microwave susceptible protein, and a qualitative model of microwave enhancement of membrane conductance is suggested.

Solution Structure of a Calmodulin-Target Peptide Complex by Multidimensional NMR

The three-dimensional solution structure of the complex between calcium-bound calmodulin (Ca^{2+}-CaM) and a 26-residue synthetic peptide comprising the CaM binding domain (residues 577 to 602) of skeletal muscle myosin light chain kinase, has been determined using multidimensional heteronuclear filtered and separated nuclear magnetic resonance spectroscopy.

Ca^{2+} Binding and Conformational Change in Two Series of Point Mutations to the Individual Ca^{2+}-binding Sites of Calmodulin

It appears that binding of Ca^{2+} to either carboxyl-terminal site can elicit the first phase of the response but the second phase is almost abolished when site 4 is the mutated site. The final conformations of site 3 and 4 mutants are thus significantly different.

Molecular Pharmacology of Calmodulin Pathways in the Cell Functions

In this paper we summarize much of the pharmacological evidence that has led to our current understanding of calmodulin-regulated cell function, with emphasis on aspects that may be relevant to drug design. These newly developed compounds are one of the most powerful tools as molecular probes for pharmacological approach, and we shed light on the physiological significance and molecular mechanisms of calmodulin-dependent pathways in various cell functions.

Signal Transduction: Regulation of cAMP Concentrationn in Cardiac Muscle by Calmodulin-Dependent Cyclic Nucleotide Phosphodiesterase

Results from this study suggest that the activity of this phosphodiesterase is precisely regulated by cross-talk between Ca^{2+} and cAMP signaling pathways. (Mol Cell Biochem 149/150: 241-247, 1995)

Analysis of the Ion Binding Sites of Calmodulin by Electrospray Ionization Mass Spectrometry

These results clearly confirm the conclusion of Milos et al. [Milos, M., Comte, M., Schaer, J.J., & Cox, J.A. (1989) *J. Inorg. Biochem. 36*, 11-25] that there should exist between four and six auxiliary sites for Ca^{2+}. Concerning the complexation of magnesium, the four proteins are able to bind two Mg^{2+} almost certainly on auxiliary cationic sites.

Spectroscopic Characterization of a High-Affinity Calmodulin-Target Peptide Hybrid Molecule

We describe the properties of a hybrid protein comprising the full length of the *Xenopus laevis* calmodulin sequence, followed by a pentapeptide linker (GGGGS), and residues 3-26 of M13, the calmodulin binding region of skeletal muscle myosin light chain kinase.

Molecular Analysis of Calmodulin and Smooth Muscle Myosin Light Chain Kinase

These facts along with a number of others have led to the postulate that the central helical region of calmodulin and troponin C is important for target protein recognition. Both chemical modifications [25,26,34-38] and genetically engineered mutants [39-43] have been utilized to emphasize the importance of this central region for enzyme recognition. The remarkable fact is that no single calmodulin variant affects all enzymes the same.

Assay of Cyclic AMP-Dependent Protein Kinases

A histone mixture is a suitable substrate for several reasons: (1) it is available from commercial sources, (2) there is little if any protein kinase contamination, (3) an adequate amount of phosphate is incorporated, (4) it is a stable and easily precipitable protein mixture, and (5) the degree of stimulation of histone phosphorylation by cAMP is usually relatively high.

Rapid Effects on Free Intracellular Calcium in Vascular Smooth Muscle and Endothelial Cells: Subcellular Localization of Calcium Elevations by Single Cell Imaging

$[Ca^{++}]i$ increase began in 30 seconds and plateaued within 5 minutes in response to physiologic concentrations of aldosterone (0.01nM-1nM), but not cortisol (10nM-10μM). $[Ca^{++}]i$ accumulated primarily around the nucleus of VSMC, but in EC peripherally, around the plasmalemma. About 15% of the VSMC exhibited a rapid transient Ca^{++} increase superimposed upon the slower steady rise seen in the EC and most VSMC.

Rapid Effects of Aldosterone on Sodium Transport in Vascular Smooth Muscle Cells

EIPA-sensitive Na^+ influx stimulation by 1nmol/L aldosterone in the presence of inhibitors of transcription and protein synthesis was almost maximal within 5 minutes, with only a small additional increase over the next 30 minutes. The induction of IP_3 by aldosterone occurred within 30 seconds, as reported in HML. Phospholipase C inhibition blocked the aldosterone effect on IP_3 generation and EIPA-sensitive Na^+ influx. The K_d for aldosterone and the mineralocorticoids fludrocortisone and deoxycorticosterone were between 0.1-0.5 nmol/L. Hydrocortisone did not cause an increase in $^{22}Na^+$ influx and IP_3 generation at doses up to 1μmol/L, nor did the mineralocorticoid receptor antagonist canrenone at 1000 fold the concentration of aldosterone block its effects.